Notes

Nucleosides. 146. 1-Methyl-5-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil, the C-Nucleoside Isostere of the Potent Antiviral Agent 1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)thymine (FMAU). Studies Directed toward the Synthesis of 2′-Deoxy-2′-Substituted Arabino Nucleosides. 6[1]

Krzysztof W. Pankiewicz,[†] Barbara Nawrot,[†] Hakan Gadler,[‡] Richard W. Price,[‡] and Kyoichi A. Watanabe*[†]

Laboratory of Organic Chemistry and Cotzias Laboratory of Neuro-Oncology, Sloan-Kettering Institute for Cancer Research, Memorial Sloan-Kettering Cancer Center, Sloan-Kettering Division of Graduate School of Medical Sciences, Cornell University, New York, New York 10021. *Received June 8, 1987*

The synthesis of 5-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1-methyluracil (**1**, C-FMAU), an isostere of the potent antiviral and antitumor nucleoside 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)thymine (2′-fluoro-5-methyl-*ara*-U or FMAU), was achieved. Pseudouridine (**2**) was converted into 4,5′-anhydro-3′-*O*-acetyl-2′-*O*-triflylpseudouridine (**4**), which was treated with tris(dimethylamino)sulfur(1+) difluorotrimethylsilicate (TASF) to give 4,5′-anhydro-5-(3-*O*-acetyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1-methyluracil (**5b**) in 40% yield. Acid hydrolysis of the 4,5′-anhydro linkage of **5b** with Dowex 50 (H^+) afforded C-FMAU. The inhibitory activity of C-FMAU against HSV-1 and HSV-2 was about 10-fold less than that of FMAU in tissue culture. This compound, however, did not show significant activity in mice inoculated with HSV-1 or HSV-2.

Our previous studies with uracil and cytosine nucleosides bearing a 2′-fluoro substituent in the "up" (arabino) configuration provided a host of potent agents against many DNA viruses.[2-6] Most notable among these are 2′-fluoro-5-iodo-*ara*-C (FIAC) and 2′-fluoro-5-methyl-*ara*-U (FMAU), both of which are effective in vitro and in vivo against herpes simplex viruses types 1 and 2 (HSV-1 and 2) and Varicella zoster virus (VZV). Both compounds inhibited human Cytomegalovirus (CMV)[4,7] as well as Epstein–Barr virus (EBV) in vitro.[8] FMAU, in addition, was shown[9] to be highly active in vivo against mouse leukemia P-815 or L-1210 made resistant to arabinosylcytosine (*ara*-C), and underwent phase 1 clinical trials.[10] More recently, the triphosphates of FIAC and FMAU have been shown[11] to be the most potent inhibitors of woodchuck hepatitis virus and human hepatitis B virus DNA polymerases in vitro. Our structure–activity relationship studies[2,3,6,12-14] showed that the 2′-fluoro substituent in the arabinosyl moiety confers far better antiviral activity than does a 2′-OH or a 2′-H. Fluorine at C-2′ was also shown to be a better choice than other halogen substituents at this locus.

The C-nucleoside 5-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1-methyluracil (**1**, 2′-fluoro-1-methyl-5-*ara*-U or C-FMAU) is an isosteric and isoelectronic isomer of FMAU and, therefore, is hoped to exhibit antiviral activity similar to that of FMAU. Though C-FMAU is expected to be less susceptible than FMAU to phosphorylation catalyzed by the viral thymidine kinase (TK) because the former is structurally a little more remote from natural thymidine than FMAU, this C-nucleoside may have a better therapeutic index because phosphorylation of C-FMAU by the TK of a normal cell would be much more difficult than that of FMAU.

For the synthesis of C-FMAU, we utilized the method recently developed in our laboratory to synthesize 2′-substituted C-nucleosides from pseudouridine[15] (**2**, Scheme I). The key intermediate is 4,5′-anhydro-1-methylpseudouridine (**3**), in which oxygen at C4 in the uracil ring is linked to C-5′ and thereby precludes its participation in nucleophilic reaction that occurs on C-2′. Anhydro-C-nucleoside **3** was regioselectively acetylated at C-3′, and then triflylated to give 3′-*O*-acetyl-4,5′-anhydro-1-methyl-2′-*O*-triflyl-pseudouridine (**4**). Although **4** had been smoothly converted into the 2′-substituted-arabinosyl

[†] Laboratory of Organic Chemistry.
[‡] Cotzias Laboratory of Neuro-Oncology.

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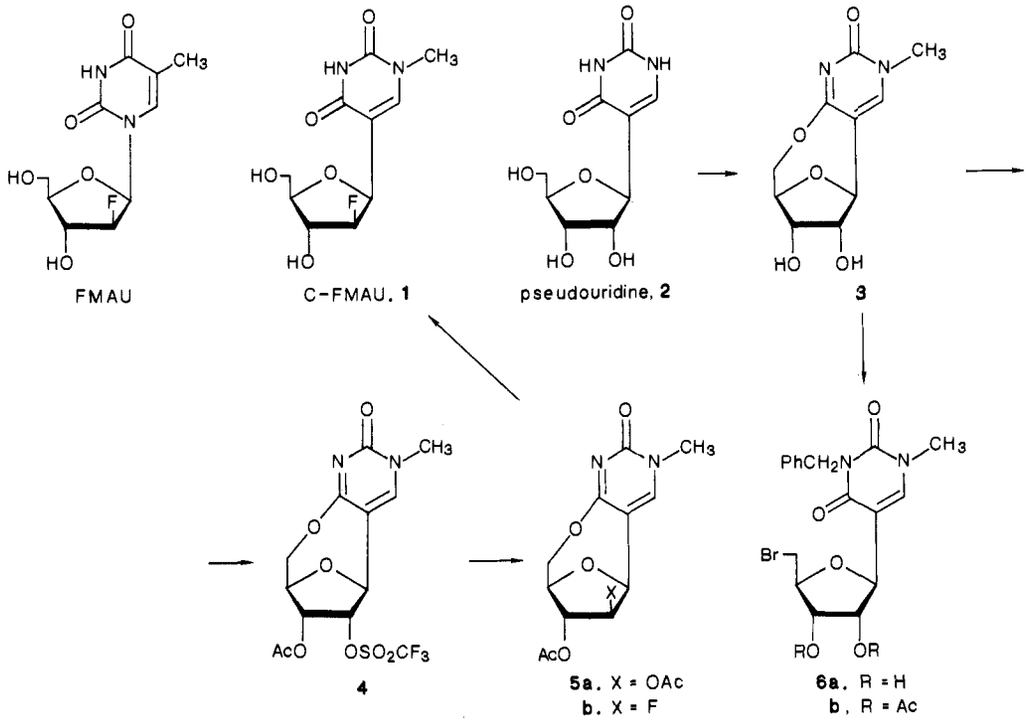
Scheme I



C-nucleosides by treatment with acetoxy, azide, chlorine, or bromine nucleophile,[15] many attempts at nucleophilic displacement of the triflate group of **4** with fluorine nucleophile including tetraalkylammonium fluoride, Amberlyst A-26 (F^-), CsF, or KF under various conditions failed. For example, treatment of **4** with Amberlyst A-26 (F^-) in acetonitrile afforded a major product, which was isolated in 15–30% yield by silica gel column chromatography. The compound was identical with 4,5′-anhydro-5-(2,3-di-*O*-acetyl-β-D-arabinofuranosyl)-1-methyluracil (**5a**), which we had synthesized previously.[15] The same product **5a** was also obtained as the major product when **4** was treated with CsF in *N*,*N*-dimethylformamide. Apparently, acetate ion that was liberated from **4** during the reaction attacked the intact **4** at C-2′ to displace the triflate to give rise to **5a**. These results indicate that an analogue of **4** in which the 3′-hydroxyl is protected by a more stable group, such as benzyl, may be converted into the 2′-fluoro arabino derivative by treatment with fluoride ion. Thus, we attempted to prepare such an analogue by treatment of **3** with di-*n*-butyltin oxide followed by benzyl bromide.[16] The tin derivative of **3**, however, did not react with benzyl bromide in *N*,*N*-dimethylformamide at room temperature. At elevated temperatures, only a less polar product was formed, which contained bromine and benzyl groups in the molecule. The ^1H NMR spectrum of this product revealed the lack of an AB quartet for H5′, 5″ characteristic for the intact 4,5′-anhydro structure. There were two exchangeable proton doublets indicating the presence of two secondary hydroxyl groups, but no exchangeable proton triplet characteristic for a primary hydroxyl and N3-H in the spectrum. These spectral data together with elemental analyses are fully consistent with the structure of 5-(5-bromo-5-deoxy-β-D-ribofuranosyl)-1-methyl-3-benzyluracil (**6a**). On acetylation of **6a**, the corresponding 2′,3′-di-*O*-acetyl derivative **6b** was formed. Finally, we found that fluorination went relatively smoothly when **4** was treated with tris(dimethylamino)sulfur(trimethylsilyl)difluoride (TASF),[17] and 4,5′-anhydro-5-(3-*O*-acetyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1-methyluracil (**5b**) was obtained in ~40% yield in pure state. Hydrolysis of the 4,5′-anhydro linkage with simultaneous removal of 3′-*O*-acetyl with Dowex 50 (H^+) afforded C-FMAU in 55% yield as a low-melting solid.

C-FMAU showed activity in vitro against HSV-1, HSV-2, and VZV. The ED_{50} values for C-FMAU were 8.5, 23, and 6.4 μM, respectively, while the values for FMAU in parallel experiments were 0.06, 0.13, and 0.02 μM, respectively, against these viruses in vitro. No morphological cytotoxicity was observed at a concentration of 1000 μM for 3 days, or a concentration of 100 μM for 9 days.

Treatment with C-FMAU did not increase the survival of HSV-1 or HSV-2 infected mice. In addition, infected animals receiving the highest doses of C-FMAU, 30 or 10 mg/kg per day, showed probable signs of neurotoxicity on day 3 and later; these animals exhibited jitter and apparent muscular spasms when handled. Neither uninfected control mice nor infected, saline-treated mice developed this effect.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Column chromatography was performed on a silica gel G60 (70–230 mesh, ASTM, Merck). Thin-layer chromatography was performed on Analtech Uniplates with short wave length UV light for vizualization. Elementary analyses were performed by M-H-W Laboratories, Phoenix, AZ, or Spang Microanalytical Laboratory, Eagle Harbor, MI. ^1H NMR spectra were recorded on a JEOL FX90Q spectrometer with Me_4Si as the internal standard. Chemical shifts are reported in ppm (δ) and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet), dt (double triplet), br s (broad singlet). Values given for coupling constants are first order.

4,5′-Anhydro-1-methylpseudouridine (3). The method of Pankiewicz et al.[15] was employed to prepare **3** from 1-methylpseudouridine[18] in 37% yield, mp 250–251 °C, undepressed upon

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admixture with an authentic sample.[15]

4,5′-Anhydro-3′-*O*-acetyl-2′-*O*-triflyl-1-methylpseudouridine (4). A mixture of **3** (1.44 g, 6 mmol) and Bu_2SnO (300 mg, 6 mmol) in MeOH (150 mL) was heated at reflux until a clear solution was obtained. The solvent was removed in vacuo, and the residue was dissolved in DMF (60 mL) and treated with Ac_2O (0.6 mL) for 3 h at room temperature. After concentration of the mixture in vacuo, the residue was triturated several times with Et_2O. The solid residue was dissolved in water (60 mL), and the solution was washed with Et_2O and concentrated in vacuo, and the residue was further dried azeotropically with toluene to give a 4:1 mixture (^1H NMR) of 3′- and 2′-acetates in quantitative yield.

A 1.5-g (5.3-mmol) sample of the above mixture was suspended in CH_2Cl_2 (200 mL). DMAP (648 mg, 5.3 mmol) and Et_3N (1.5 mL, 10.6 mmol) were added to the suspension followed by triflyl chloride (1.2 mL, 10.6 mmol). The mixture was stirred at room temperature for 1 h and concentrated in vacuo, and the residue was chromatographed on a silica gel column ($CHCl_3$–EtOH, 49:1, v/v) to give **4** (1.8 g, 75%), mp 135–136 °C dec [lit.[15] mp 130–135 °C dec].

4,5′-Anhydro-5-(2,3-di-*O*-acetyl-β-D-arabinofuranosyl)-1-methyluracil (5a). A mixture of **4** (35 mg, 0.08 mmol) and CsF (30 mg) in DMF (1 mL) was heated at 90 °C for 24 h and then concentrated in vacuo. The residue was dissolved in $CHCl_3$ (10 mL), washed with water (2 × 2 mL), and dried (Na_2SO_4). The solvent was removed in vacuo, and the residue was purified on a silica gel column with $CHCl_3$–EtOH (9:1, v/v) as the eluent to give **5a** (8 mg, 30%). The IR and ^1H NMR spectra of this product were identical with those of **5a** prepared earlier.[15] In a similar manner, treatment of **4** with Amberlyst A-26 (F^-) in CH_2Cl_2 (refluxing for 48 h) afforded **5a** in ~15% yield.

4,5′-Anhydro-5-(3-*O*-acetyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1-methyluracil (5b). To a solution of **4** (150 mg, 0.36 mmol) in dry CH_2Cl_2 (2 mL) was added a solution of TASF[17] (300 mg, 1.08 mmol) in dry CH_2Cl_2 in an atmosphere of argon at −70 °C. After the mixture was stirred at −72 °C for 30 min, a second charge of TASF (200 mg, 0.72 mmol) was added. The mixture was allowed to warm to room temperature, stirring was continued for 2 h, and then the reaction was quenched by addition of water (0.5 mL). The organic layer was separated, washed with water (0.5 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed on a silica gel column ($CHCl_3$–EtOH, 95:5, v/v) to give **5b** (40 mg, 38.8% after recrystallization from Et_2O), mp 270–274 °C. ^1H NMR (DMSO-d_6): δ 2.11 (s, 3 H, Ac), 3.88 (s, 3 H, Me), 4.08 (d, 1 H, H-5′, $J_{4',5'}$ = 0, $J_{5',5''}$ = 13.2 Hz), 4.40 (m, 1 H, H-4′), 4.57 (dd, 1 H, H-5″, $J_{4',5''}$ = 2.75, $J_{5',5''}$ = 13.1 Hz), 5.26 (dd, 1 H, H-3′, $J_{3',F}$ = 18.66 Hz), 5.31 (dd, 1 H, H-1′, $J_{1',2'}$ = 8.23, $J_{1',F}$ = 17.29 Hz), 5.35 (dd, 1 H, H-2′, $J_{1',2'}$ = 8.23, $J_{2',F}$ = 53.0 Hz), 8.24 (s, 1 H, H-6). ^{19}F NMR (DMSO-d_6): δ −76.8 (in reference to $CFCl_3$) (dd, $J_{F,2'}$ = 53.7, $J_{F,3'}$ = 18.6 Hz). ^{13}C NMR (DMSO-d_6): δ 172.6 (s, C-2), 170.1 (s, C-4), 151.5 (s, C-6), 104.0 (d, C-5), 93.3 (d, C-2′, $J_{F,C2'}$ = 181.6 Hz), 80.0 (d, C-4′, $J_{F,C4'}$ = 6.1 Hz), 78.8 (d, C-1′, $J_{F,C1'}$ = 29.3 Hz), 75.1 (d, C-3′, $J_{F,C3'}$ = 21.9 Hz), 73.8 (s, C-5′), 41.3–36.6 (m, DMSO-d_6 and 1-Me), 2.05 (s, Ac). Anal. Calcd for $C_{12}H_{13}FN_2O_5$: C, 50.70; H, 4.58; F, 6.69; N, 9.36. Found: C, 50.31; H, 4.57; F, 6.75; N, 9.57. MS, (*m/e*): 285 (MH^+).

5-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-1-methyluracil (C-FMAU, 1). A mixture of **5b** (285 mg, 1 mmol) and Dowex 50 (H^+) (5 mL) in water (50 mL) was stirred for 3 h at 70 °C. The resin was removed by filtration and washed with water, and the combined filtrate and washings were concentrated in vacuo. The residue was chromatographed on a silica gel column ($CHCl_3$–EtOH, 9:1, v/v) to give **1** (136 mg, 55%) as a syrup, which crystallized upon standing at room temperature, mp 65–67 °C. ^1H NMR (DMSO-d_6): δ 3.28 (s, 3 H, NMe), 3.50 (m, 2 H, H-5′, 5″), 3.71 (m, 1 H, H-4′), 4.12 (dt, 1 H, H-3′, became dd upon addition of D_2O, $J_{2',3'}$ = 0, $J_{3',4'}$ = 3.0, $J_{3',F}$ = 17.0 Hz), 4.80 (t, 1 H, exchangeable, CH_2OH), 4.83 (dd, 1 H, H-1′, $J_{1',2'}$ = 2.5, $J_{1',F}$ = 29.64 Hz), 4.92 (dd, 1 H, H-2′, $J_{1',2'}$ = 2.5, $J_{2',3'}$ = 0, $J_{2',F}$ = 49.12 Hz), 5.63 (d, 1 H, OH), 7.53 (d, 1 H, H-6, collapsed to a singlet upon addition of D_2O). ^{19}F NMR (DMSO-d_6): δ −73.32 (octet, $J_{2',F}$ = 52.2, $J_{1',F}$ = 17.6, $J_{3',F}$ = 28.8 Hz). ^{13}C NMR (DMSO-d_6): δ 162.8 (s, C-2), 151.1 (s, C-4), 143.7 (s, C-6), 107.8 (d, C-5, $J_{F,C5}$ = 4.9 Hz), 96.9 (d, C-2′, $J_{F,C2'}$ = 185.5 Hz), 85.6 (s, C-4′), 76.1 (d, C-1′, $J_{F,C1'}$ = 9.8 Hz), 75.1 (d, C-3′, $J_{F,C3'}$ = 2.4 Hz), 61.5 (s, C-5′), 35.7 (s, NMe). Anal. Calcd for $C_{10}H_{13}FN_2O_5$: C, 46.15; H, 5.03; F, 7.10; N, 10.77. Found: C, 46.04; H, 5.27; F, 7.12; N, 10.52.

5-(5-Bromo-5-deoxy-β-D-ribofuranosyl)-3-benzyl-1-methyluracil (6a). A mixture of **3** (720 mg, 3 mmol) and Bu_2SnO(750 mg) in MeOH (30 mL) was heated under reflux until a clear solution was obtained. The mixture was concentrated, the residue was dissolved in DMF, and benzyl bromide (0.1 mL) was added. After being stirred for 3 h at room temperature, the mixture was heated at 100 °C for 1 h. The mixture was concentrated in vacuo, and the residue was chromatographed over a silica gel column ($CHCl_3$–EtOH, 33:1, v/v) to give **6a** (240 mg) as a foam. ^1H NMR (DMSO-d_6): δ 3.32 (s, 3 H, NMe), 3.69–4.10 (m, 5 H, H-2′, 3′, 4′, 5′, 5″), 4.57 (d, 1 H, H-1′, $J_{1',2'}$ = 3.8 Hz), 4.98 (s, 2 H, CH_2Ph), 5.00 (d, 1 H, OH), 5.10 (d, 1 H, OH), 7.27 (s, 5 H, Ph), 7.72 (s, 1 H, H-6). Anal. Calcd for $C_{17}H_{14}BrN_2O_5$: C, 49.65; H, 4.65; Br, 19.43; N, 6.81. Found: C, 50.12; H, 4.71; Br, 19.06; N, 6.90.

5-(2,3-Di-*O*-acetyl-5-bromo-5-deoxy-β-D-ribofuranosyl)-3-benzyl-1-methyluracil (6b). Acetylation of **6a** with Ac_2O in pyridine afforded, after concentration of the mixture in vacuo and several coevaporations of the residue with toluene and EtOH, crystalline **6b** in quantitative yield, mp 118–119 °C. ^1H NMR (DMSO-d_6): δ 2.03 (s, 6 H, 2 Ac), 3.32 (s, 3 H, NMe), 3.67–3.74 (m, 2 H, H-5′, 5″), 4.12–4.18 (m, 1 H, H-4′), 4.77 (d, 1 H, H-1′, $J_{1',2'}$ = 4.9 Hz), 4.98 (s, 2 H, CH_2Ph), 5.19–5.42 (m, 2 H, H-2′, 3′), 7.27 (s, 5 H, Ph), 7.87 (s, 1 H, H-6). Anal. Calcd for $C_{21}H_{23}BrN_2O_7$: C, 50.92; H, 4.68; Br, 16.13; N, 5.65. Found: C, 50.96; H, 4.72; Br, 15.98; N, 5.51.

HSV Infection in Vitro. The antiviral efficacy of C-FMAU was assessed in a microtiter assay as previously described.[19] Briefly, 10-fold dilutions of C-FMAU were tested against HSV-1, HSV-2, and VZV in human foreskin fibroblasts. The medium was replenished every 3 days, and the ED_{50} of the drug was determined by scoring the inhibition of cytopathic effect. Toxicity of the drug was assessed by observing uninfected cell monolayers.

Animal Model of HSV Infection. Female Balb/c mice, body weight approximately 15–16 g were infected with HSV-1 or HSV-2. Animals were infected either intraocularly (HSV-1, strain SC-16) or intracerebrally (HSV-2, strain G). The virus dilutions were chosen so that approximately 50% of the animals were killed by day 8. Intraocular injections were given in 4 μL of saline, intracerebral injections in 20 μL of saline. Control animals received only saline. All mice were injected intraperitoneally twice daily with C-FMAU at concentrations ranging from 30 mg/kg per day to 0.1 mg/kg per day or with saline only, in a volume of 100 μL. Treatment began 3 days after intraocular infection or approximately 6 h after intracerebral infection and was continued for 5 days.

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Registry No. 1, 110419-25-5; **3**, 97416-19-8; **3** (3′-acetate), 97416-31-4; **3** (2′-acetate), 97416-32-5; **4**, 97430-85-8; **5a**, 97416-23-4; **5b**, 110419-24-4; **6a**, 110419-26-6; **6b**, 110419-27-7.

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